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Substitute Form PTO 1390 U.S. Department of Commerce Patent and Trademark Office		Attorney's Docket Number: 50186/003001
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. Application Number: Not known yet
INTERNATIONAL APPLICATION NUMBER	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP99/03071	05 May 1999 (05.05.99)	8 May 1998 (08.05.98)
TITLE OF INVENTION:		HUMAN DEADENYLATING NUCLEASE, ITS PRODUCTION AND ITS USE
APPLICANTS FOR DO/EO/US:		Christoph Hüls et al.
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:		
1.	<input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.	
2.	<input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.	
3.	<input checked="" type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).	
4.	<input type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19 <sup>th</sup> month from the earliest claimed priority date.	
5.	A copy of the International Application as filed (35 U.S.C. 371(c)(2)). <input checked="" type="checkbox"/> a. is transmitted herewith (required only if not transmitted by the International Bureau). <input type="checkbox"/> b. has been transmitted by the International Bureau. <input type="checkbox"/> c. is not required, as the application was filed with the United States Receiving Office (RO/US).	
6.	<input checked="" type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)).	
7.	Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)). <input checked="" type="checkbox"/> a. are transmitted herewith (required only if not transmitted by the International Bureau). <input type="checkbox"/> b. have been transmitted by the International Bureau. <input type="checkbox"/> c. have not been made; however, the time limit for making such amendments has NOT expired. <input type="checkbox"/> d. have not been made and will not be made.	
8.	<input checked="" type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).	
9.	<input type="checkbox"/> An oath or declaration of the inventors (35 U.S.C. 371(c)(4)).	
10.	<input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).	
11.	<input checked="" type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.	
12.	<input type="checkbox"/> An assignment for recording. A separate cover sheet in compliance with 37.3.28 and 3.31 is included.	
13.	<input checked="" type="checkbox"/> A FIRST preliminary amendment. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.	
14.	<input type="checkbox"/> A substitute specification.	

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15.	<input type="checkbox"/> A change of power of attorney and/or address letter.																																																																												
16.	<input type="checkbox"/> Other items or information:																																																																												
17.	<p><input checked="" type="checkbox"/> The following fees are submitted:</p> <p>BASIC NATIONAL FEE (37 CFR 1.492(A)(1)-(5)):</p> <table> <tr> <td>Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.455(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO</td> <td>\$ 970.00</td> </tr> <tr> <td>International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO</td> <td>\$ 840.00</td> </tr> <tr> <td>International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO</td> <td>\$ 690.00</td> </tr> <tr> <td>International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1) - (4)</td> <td>\$ 670.00</td> </tr> <tr> <td>International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)</td> <td>\$ 96.00</td> </tr> </table> <p>ENTER APPROPRIATE BASIC FEE AMOUNT = \$ 840.00</p> <p>Surcharge of \$130 for furnishing the oath or declaration later than <input type="checkbox"/> 20 OR <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).</p> <table> <thead> <tr> <th>CLAIMS</th> <th>NUMBER FILED</th> <th>NUMBER EXTRA</th> <th>RATE</th> </tr> </thead> <tbody> <tr> <td>Total claims</td> <td>39 - 20 =</td> <td>19</td> <td>x \$18.00</td> <td>\$ 342.00</td> </tr> <tr> <td>Independent claims</td> <td>3 - 3 =</td> <td>0</td> <td>x \$80.00</td> <td>\$</td> </tr> <tr> <td colspan="3">Multiple dependent claims (if applicable)</td> <td>+ \$280.00</td> <td>\$ 280.00</td> </tr> <tr> <td colspan="4">TOTAL OF ABOVE CALCULATIONS =</td> <td>\$ 1462.00</td> </tr> <tr> <td colspan="4">Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed with this request (Note 37 CFR 1.9, 1.27, 1.28).</td> <td>\$</td> </tr> <tr> <td colspan="4">SUBTOTAL =</td> <td>\$</td> </tr> <tr> <td colspan="4">Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 OR <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).</td> <td>+ \$</td> </tr> <tr> <td colspan="4">TOTAL NATIONAL FEE =</td> <td>\$</td> </tr> <tr> <td colspan="4">Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property.</td> <td>+ \$</td> </tr> <tr> <td colspan="4">TOTAL FEES ENCLOSED =</td> <td>\$1462.00</td> </tr> <tr> <td colspan="4"></td> <td>Amount to be refunded \$</td> </tr> <tr> <td colspan="4"></td> <td>charged \$</td> </tr> </tbody> </table> <p><input checked="" type="checkbox"/> a. A check in the amount of \$1462.00 to cover the above fees is enclosed.  <input type="checkbox"/> b. Please charge my Deposit Account No. 03-2095 in the amount of \$ [**.**] to cover the above fees.  <input checked="" type="checkbox"/> c. The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Deposit Account No. 03-2095.</p>			Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.455(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO	\$ 970.00	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO	\$ 840.00	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO	\$ 690.00	International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1) - (4)	\$ 670.00	International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)	\$ 96.00	CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	Total claims	39 - 20 =	19	x \$18.00	\$ 342.00	Independent claims	3 - 3 =	0	x \$80.00	\$	Multiple dependent claims (if applicable)			+ \$280.00	\$ 280.00	TOTAL OF ABOVE CALCULATIONS =				\$ 1462.00	Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed with this request (Note 37 CFR 1.9, 1.27, 1.28).				\$	SUBTOTAL =				\$	Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 OR <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				+ \$	TOTAL NATIONAL FEE =				\$	Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property.				+ \$	TOTAL FEES ENCLOSED =				\$1462.00					Amount to be refunded \$					charged \$
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NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b) must be filed and granted to restore the application to pending status.

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Revised: 17 March 2000

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Christoph Hüls et al.

Art Unit:

Serial No.: Not yet assigned

Examiner:

Filed: October 25, 2000

Title: HUMAN DEADENYLATING NUCLEASE, ITS PRODUCTION AND  
ITS USE

Commissioner For Patents  
Washington, DC 20231

PRELIMINARY AMENDMENT

Prior to examination, kindly amend the above-referenced application as follows.

In the claims:

Replace current claims 1-18 with the following new claims 19-42.

--19. A nucleic acid which encodes a human deadenylating nuclease (DAN)

having an amino acid sequence as depicted in SEQ ID NO:11 or a functional variant thereof, and fragments thereof having at least 8 nucleotides.

20. A nucleic acid as claimed in claim 19, wherein the nucleic acid is a DNA or RNA.

21. A nucleic acid as claimed in claim 20, wherein the nucleic acid is a double-stranded DNA.

22. A nucleic acid as claimed in claim 19, wherein the nucleic acid is a DNA having a nucleic acid sequence as depicted in SEQ ID NO:12 from position 58 to 1977.

23. A nucleic acid as claimed in claim 22, wherein the nucleic acid contains one or more noncoding sequences and/or a polyA sequence.

24. A nucleic acid as claimed in claim 19, wherein the nucleic acid is contained in a vector.

25. A nucleic acid as claimed in claim 24, wherein the nucleic acid is contained in an expression vector.

26. A nucleic acid as claimed in claim 24, wherein the nucleic acid is contained in a vector which is effective in gene therapy.

27. A process for preparing a nucleic acid which encodes a human deadenylating nuclease (DAN) having an amino acid sequence as depicted in SEQ ID NO: 11 or a functional variant thereof or a fragment thereof having at least 8 nucleotides, said process comprising chemically synthesizing said nucleic acid or isolating said nucleic acid from a gene library using a probe.

28. A polypeptide having an amino acid sequence as depicted in SEQ ID NO: 11 or a functional variant thereof, and fragments thereof having at least 6 amino acids.

29. A process for preparing a polypeptide having an amino acid sequence as depicted in SEQ ID NO:11 or a functional variant thereof, and fragments thereof having at least 6 amino acids, said process comprising expressing a nucleic acid of claim 19 in a suitable host cell.

30. An antibody directed against a polypeptide as claimed in claim 28.

31. A process for preparing an antibody as claimed in claim 30, said process comprising immunizing a mammal with a polypeptide as claimed in claim 28 and optionally isolating the resulting antibody.

32. A pharmaceutical composition comprising a nucleic acid as claimed in claim 19 or a polypeptide as claimed in claim 28 and, optionally, pharmaceutically acceptable additives and/or adjuvants.

33. A process for producing a pharmaceutical for treating cancer, an autoimmune disease, Alzheimer's disease, an allergy, arthrosis, atherosclerosis, osteoporosis, an acute or chronic infectious disease, or diabetes, or for influencing the metabolism of a cell, said process comprising formulating a nucleic acid as claimed in claim 19, a polypeptide as claimed in claim 28, or an antibody as claimed in claim 30 together with a pharmaceutically acceptable additive and/or adjuvant.

34. The process of claim 33, wherein the autoimmune disease is multiple sclerosis or rheumatoid arthritis; the allergy is neurodermatitis, a type I allergy, or a type IV allergy; or the cell metabolism is associated with immunosuppression or transplantation.

35. A diagnostic agent which comprises a nucleic acid as claimed in claim 19, a polypeptide as claimed in claim 28, or an antibody as claimed in claim 30 and, optionally, suitable additives and/or adjuvants.

36. A process for producing a diagnostic agent for diagnosing cancer, an

autoimmune disease, Alzheimer's disease, an allergy, arthrosis, atherosclerosis, osteoporosis, an acute or chronic infectious disease, or diabetes, or for analyzing the metabolism of a cell, said process comprising adding a pharmaceutically acceptable excipient to a nucleic acid as claimed in claim 19, a polypeptide as claimed in claim 28, or an antibody as claimed in claim 30.

37. The process of claim 36, wherein the autoimmune disease is multiple sclerosis or rheumatoid arthritis; the allergy is neurodermatitis, a type I allergy, or a type IV allergy; or the cell metabolism is associated with immune status or transplantation status.

38. A test for identifying functional interactors, comprising a nucleic acid as claimed in claim 19, a polypeptide as claimed in claim 28, or an antibody as claimed in claim 30 and, optionally, suitable additives and/or adjuvants.

39. A process for identifying functional interactors of DAN, said process comprising contacting a sample with a nucleic acid as claimed in claim 19 or a polypeptide as claimed in claim 28, and isolating said interactor.

40. A process of finding variants of human DAN, said process comprising screening a gene library with a nucleic acid as claimed in claim 19 and isolating nucleic

acid variants.

41. A process for poly(A)-specific degradation of nucleic acids, said process comprising contacting a nucleic acid with a polypeptide as claimed in claim 28.

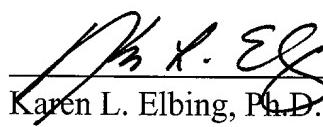
42. A process as claimed in claim 41, wherein the nucleic acid is mRNA.--

Conclusion

If there are any charges, or any credits, kindly apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: 25 October 2000

  
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\Ntserver\documents\50186\50186.003001 Preliminary Amendment for the Claims.wpd

## Human deadenylating nuclease, its preparation and use

## Description

- 5 The present invention relates to a human deadenylating nuclease (DAN), to its coding nucleic acid and to its preparation and use.

The intracellular concentration of mRNA appears to be controlled by its rate of degradation as well as by its rate of production. In this connection, 10 mRNAs appear not to be degraded by a random nucleolytic event but, rather, by specific mechanisms and by rates of degradation which are specific for the given RNA. Currently, two different degradation pathways are known in which the poly(A) tails play a particular role. In *E. coli*, poly(A) tails are appended to mRNAs or mRNA fragments which are generated by 15 RNase E. The poly(A) tail appears to function as a relatively unstructured unit which is responsible for the attachment of a complex which, in addition to other proteins, contains a progressive 3'-exonuclease, which is a polynucleotide phosphorylase, and an RNA-dependent ATPase which helps the exonuclease to circumvent inhibitory secondary structures in the 20 mRNA. A similar mechanism appears to operate in chloroplasts.

In eukaryotes, exonucleolytic truncation of the poly(A) tail likewise initiates degradation of many but not all mRNAs. In contrast to the process in bacteria, the poly(A)-degrading exonuclease does not appear, in carrying 25 out its degradation, to continue into the 3'-UTR and the coding sequence. Instead, after having degraded the poly(A) tail, the eukaryotic exonuclease appears to stop. In *S. cerevisiae*, the second step of mRNA degradation involves removal of the 5' cap structure by a specific pyrophosphatase. However, the CAP is only removed after the poly(A) tail has been 30 shortened down to approx. 10-15 nucleotides. Removal of the CAP structure makes the mRNA accessible to 5'-exonucleases, whose most important representative is encoded by the XRN1 gene.

While it is relatively easy to observe the initial deadenylation process in 35 mammalian cells, investigation of the additional steps is rendered more difficult by the rapidity of the subsequent degradation processes and the absence of analyzable intermediates. However, indirect evidence suggests that the CAP structure is removed in the second step. A homolog of the

- XRN1 gene has been described in the mouse. In general, stable mRNAs are deadenylated slowly and unstable mRNAs are deadenylated rapidly. Rapid degradation depends on the presence of particular destabilizing sequences in the 3'-UTR or in the coding sequence. Furthermore,
- 5 mutations in these sequences lead not only to the mRNA being stabilized but also to deadenylation being slowed down. By contrast, inactivation of a stabilizing element in  $\alpha$ -globulin mRNA leads to instability and to accelerated deadenylation. These data provide clear support for degradation of mRNA being controlled by the deadenylation rate.
- 10 The deadenylation of particular mRNAs can lead to inactivation of translation as well. This can be explained by the importance of the poly(A) tail for the initiation of translation. Deadenylation as a mechanism for regulating translation plays a crucial role in the maturation of oocytes and
- 15 in early embryogenesis in many animal species. For example, in Drosophila, the polarity of the embryo is regulated by deadenylation of the so-called hunchback mRNA.
- In vertebrates, three deadenylation reactions can in principle be distinguished in oocyte maturation and early embryogenesis:
- 20 1. In immature oocytes, most mRNAs are stored in a translationally inactive form in which they possess short poly(A) tails. An example of this is the mRNA for tPA (tissue plaminogen activator) in mice. This mRNA is initially given a long poly(A) tail in the normal
- 25 polyadenylation reaction, with this tail then being truncated by deadenylation into an oligo(A) tail. This truncation is regulated by sequences in the 3'-UTR.
- 30 2. During maturation of oocytes, deadenylation is used to inactivate particular mRNAs which originally had long poly(A) tails and were active in early egg development. The deadenylation does not depend on specific sequences in the mRNA. All mRNAs are deadenylated unless they are protected by an active adenylation process.
- 35 3. During early embryogenesis, certain mRNAs are deadenylated in a specific reaction which likewise requires specific sequences in the 3'-UTR.

All three deadenylation reactions takes place in the cytoplasm. However, in contrast to the situation in somatic cells, the oligoadenylated or

deadenylation mRNAs remain stable in oocytes and are either adenylated once again or only degraded in subsequent development stages.

The enzymes which are responsible for these reactions have so far not  
5 been identified unambiguously. It has been shown in yeast that  
degradation of poly(A) depends directly or indirectly on the poly(A)-binding  
protein (PAB1). Although a PAB1-dependent poly(A)-nuclease (PAN) has  
been purified (Sachs, A.B. & Deardorff (1992) Cell, 70, 961; Lowell, J.E. et  
al. (1992) Genes Dev., 6, 2088), it has only been possible to identify minor  
10 defects in deadenylation in mutants of the genes concerned (PAN2 and  
PAN3) (Boeck, R. et. Al. (1996) 271 (1), 432; Brown, C. et al. (1996) 16  
(10) 5744).

Körner, Ch. G. & Wahle, E. (1997) J. Biol. Chem., 272, 10448, No. 16  
15 describe an Mg<sup>2+</sup>-dependent poly(A)-specific 3'-exoribonuclease from calf  
thymus which has a molecular weight of 74 kDa and which has also been  
referred to as being a deadenylating nuclease (DAN). The calf thymus  
DAN which has been described is stimulated by the cytoplasmic poly(A)-  
binding protein I (PAB I) under defined reaction conditions (Körner, Chr. &  
20 Wahle, E. (1997), see above) and preferentially poly(A) as its substrate.

The object of the present invention was to make available a human  
poly(A)-specific 3'-exoribonuclease.

25 Surprisingly, a clone which encodes a human deadenylating nuclease  
(human DAN) in accordance with the present invention, but which had not  
been characterized in detail and which had only been sequenced  
terminally, has now been found in a gene library.  
  
30 The present invention therefore relates to a nucleic acid which encodes a  
human DAN having an amino acid sequence as depicted in SEQ 11 or a  
functional variant thereof, and parts thereof having at least 8 nucleotides,  
preferably having at least 15 or 20 nucleotides, in particular having at least  
100 nucleotides, very particularly having at least 300 nucleotides  
35 (subsequently termed "nucleic acid according to the invention").

The complete nucleic acid encodes a protein having 639 amino acids and  
a molecular mass of 73.5 kDa. Expression of the nucleic acid in E. coli  
gave rise to a protein which displays enzymic activities which are similar to

- those displayed by the DAN described by Körner, Ch. G. & Wahle, E. (1997, see above). Other experiments performed in accordance with the present invention confirmed that the nucleic acid is a nucleic acid which encodes a human DAN. The nucleic acid according to the invention was
- 5 deposited in the DSMZ - Deutsche Sammlung von Mikroorganismen und Zellkulturen [German collection of microorganisms and cell cultures] GmbH, Mascheroder Weg 1b, 38124 Brunswick under number DSM 12075 on March 25, 1998.
- 10 In a preferred embodiment, the nucleic acid according to the invention is a DNA or RNA, preferably a double-stranded DNA, and in particular a DNA having a nucleic acid sequence as depicted in SEQ 12 from pos. 58 to pos. 1977. The two positions determine, according to the present invention, the beginning and the end of the coding region.
- 15 According to the present invention, the term "functional variant" is understood as meaning a nucleic acid which is functionally related to the human DAN-encoding nucleic acid and is, in particular, of human origin. Examples of related nucleic acids are nucleic acids from different human
- 20 cells or tissues, or allelic variants. The present invention likewise encompasses variants of nucleic acids which can be derived from different human individuals.
- In the broader sense, the term "variants" is understood, according to the
- 25 present invention, as meaning nucleic acids which exhibit a homology, in particular a sequence identity, of approx. 60%, preferably of approx. 75%, in particular of approx. 90% and, very particularly, of approx. 95%.
- The parts of the nucleic acid according to the invention can, for example,
- 30 be used for preparing individual epitopes, as probes for identifying other functional variants or as antisense nucleic acids. For example, a nucleic acid composed of at least approx. 8 nucleotides is suitable for use as an antisense nucleic acid, a nucleic acid composed of at least approx. 15 nucleotides is suitable for use as a primer in the PCR method, a nucleic
- 35 acid composed of at least approx. 20 nucleotides is suitable for identifying further variants, and a nucleic acid composed of at least approx. 100 nucleotides is suitable for use as a probe.

In another preferred embodiment, the nucleic acid according to the invention contains one or more noncoding sequences and/or a poly(A) sequence. The noncoding sequences are, for example, intron sequences or regulatory sequences, such as promoter or enhancer sequences, for  
5 regulated expression of the human DAN-encoding gene.

In another embodiment, the nucleic acid according to the invention is therefore contained in a vector, preferably in an expression vector or a vector which is effective in gene therapy.  
10

The expression vectors can, for example, be prokaryotic or eukaryotic expression vectors. Examples of prokaryotic expression vectors for expression in *E. coli* are the T7 expression vector pGM10 (Martin, 1996), which encodes an N-terminal Met-Ala-His6 tag, which enables the  
15 expressed protein to be advantageously purified by way of an Ni<sup>2+</sup>-NTA column. Examples of eukaryotic expression vectors which are suitable for expression in *Saccharomyces cerevisiae* are the vectors p426Met25 and p426GAL1 (Mumberg et al. (1994) Nucl. Acids Res., 22, 5767), while examples of such vectors which are suitable for expression in insect cells  
20 are baculovirus vectors as disclosed in EP-B1-0127839 or EP-B1-0549721, and for expression in mammalian cells are SV40 vectors, with these vectors being generally available.

In general, the expression vectors also contain regulatory sequences which  
25 are suitable for the host cell, such as the trp promoter for expression in *E. coli* (see, e.g., EP-B1-0154133), the ADH-2 promoter for expression in yeasts (Russel et al., (1983), J. Biol. Chem. 258, 2674), the baculovirus polyhedrin promoter for expression in insect cells (see, e.g., EP-B1-0127839) or the early SV40 promoter or LTR promoters, e.g. from MMTV  
30 (mouse mammary tumor virus; Lee et al. (1981) Nature, 214, 228).

Examples of vectors which are effective in gene therapy are virus vectors, preferably adenovirus vectors, in particular replication-deficient adenovirus vectors or adenoassociated virus vectors, e.g. an adenoassociated virus  
35 vector which is composed solely of two inserted terminal repeat (ITR) sequences.

Examples of suitable adenovirus vectors are described in McGrory, W.J. et al. (1998) Virol. 163, 614; Gluzman, Y. et al. (1982) in "Eukaryotic Viral

Vectors" (Gluzman, Y. ed.) 187, Cold Spring Harbor Press, Cold Spring Harbor, New York; Chroboczek, J. et al. (1992) Virol. 186, 280; Karlsson, S. et al. (1986) EMBO J., 5, 2377 or WO 95/00655.

- 5 Suitable adenoassociated virus vectors are described, for example, in  
Muzychka, N. (1992) Curr. Top. Microbiol. Immunol. 158, 97; WO  
95/23867; Samulski, R.J. (1989) J. Virol. 63, 3822; WO 95/23867; Chiorini,  
J.A. et al. (1995) Human Gene Therapy 6, 1531 or Kotin, R.M. (1994)  
Human Gene Therapy 5, 793.

10

- Vectors which are effective in gene therapy can also be obtained by  
complexing the nucleic acid according to the invention with liposomes.  
Lipid mixtures as described in Felgner, P.L. et al. (1987) Proc. Natl. Acad.  
Sci, USA 84, 7413; Behr, J.P. et al. (1989) Proc. Natl. Acad. Sci. USA 86,  
15 6982; Felgner, J.H. et al. (1994) J. Biol. Chem. 269, 2550 or Gao, X. &  
Huang, L. (1991) Biochim. Biophys. Acta 1189, 195 are suitable for this  
purpose. When the liposomes are being prepared, the DNA is bonded  
ionically to the surface of the liposomes in a ratio which is such that a  
positive net charge remains and the DNA is completely complexed by the  
liposomes.

- 20 The nucleic acid according to the invention can, for example, be  
synthesized chemically, e.g. by the phosphotriester method (see, e.g.,  
Uhlman, E. & Peyman, A. (1990) Chemical Reviews, 90, 543, No. 4), either  
25 on the basis of the sequence disclosed in SEQ 12 or on the basis of the  
peptide sequence disclosed in SEQ 11 and making use of the genetic  
code. Another possibility of obtaining the nucleic acid according to the  
invention is that of using a suitable probe (see, e.g., Sambrook, J. et al.  
30 (1989) Molecular Cloning. A laboratory manual. 2nd Edition, Cold Spring  
Harbor, New York) to isolate it from a suitable gene library, for example  
from a human gene library. Examples of suitable probes are single-  
stranded DNA fragments which are of a length of from approx. 100 to  
1000 nucleotides, preferably of a length of from approx. 200 to 500  
nucleotides, in particular of a length of from approx. 300 to 400  
35 nucleotides, and whose sequence can be derived from the nucleic acid  
sequence depicted in SEQ 12.

The present invention furthermore also relates to the polypeptide itself  
having an amino acid sequence as depicted in SEQ 11 or a functional

variant thereof, and parts thereof having at least six amino acids, preferably having at least 12 amino acids, in particular having at least 65 amino acids and very particularly having 638 amino acids (subsequently termed "polypeptide according to the invention"). For example, a 5 polypeptide which is approx. 6-12 amino acids in length, preferably approx. 8 amino acids in length, can contain an epitope which, after having been coupled to a carrier, is used for preparing specific polyclonal or monoclonal antibodies (in this regard, see, e.g., US 5,656,435). Polypeptides whose 10 length is at least approx. 65 amino acids can also be used directly, without any carrier, for preparing polyclonal or monoclonal antibodies.

Within the meaning of the present invention, the term "functional variant" is understood as denoting polypeptides which are functionally related to the peptide according to the invention, i.e. which exhibit a poly(A)-specific 3'-exoribonuclease activity and which are preferably active under two different reaction conditions. In the first reaction condition, the activity is completely dependent on the presence of spermidine when salt is absent. By contrast, the activity of the enzyme is dependent on the salt concentration when spermidine is absent. Furthermore, it is possible to observe stepwise degradation of the poly(A) tail under defined reaction conditions when PAB1 is present (see also Körner, Chr. G. & Wahle, E. (1997), see above). In particular, the lengths of the predominant degradation products differ by approx. 30 nucleotides. Variants are also understood as meaning allelic variants or polypeptides which are derived from other human cells or tissues. They are also understood as meaning polypeptides which are derived from different human individuals.

In the broader sense, they are also understood as meaning polypeptides which possess a sequence homology, in particular a sequence identity, with the polypeptide having the amino acid sequence depicted in SEQ 11 of approx. 70%, preferably of approx. 80%, in particular of approx. 90%, very particularly of approx. 95%. They furthermore also include deletion of the polypeptide in the range of approx. 1 - 60, preferably of approx. 1 - 30, in particular of approx. 1 - 15, very particularly of approx. 1 - 5 amino acids.

For example, the first amino acid, i.e. methionine, can be missing without the function of the polypeptide being significantly altered. In addition, they also include fusion proteins which contain the above-described polypeptides according to the invention, with the fusion proteins already themselves possessing the function of a human DAN or only being able to

acquire the specific function after the fusion moiety has been eliminated. Very particularly, they include fusion proteins having a content of, in particular, nonhuman sequences of approx. 1 - 200, preferably approx. 1 - 150, in particular of approx. 1 - 100, very particularly of approx. 1 - 50

5 amino acids. Examples of nonhuman peptide sequences are prokaryotic peptide sequences, for example from *E. coli* galactosidase or a so-called histidine tag, e.g. a Met-Ala-His<sub>6</sub> tag. A fusion protein containing a so-called histidine tag is particularly advantageously suitable for purifying the expressed protein by way of metal ion-containing columns, for example by 10 way of an Ni<sup>2+</sup>-NTA column. "NTA" stands for the chelating agent nitrilotriacetic acid (Qiagen GmbH, Hilden).

The parts of the polypeptide according to the invention represent, for example, epitopes which can be specifically recognized by antibodies.

15 By comparing with known nucleases, it was found that the polypeptide according to the invention is a member of the RNaseD family. Figure 4 shows the conserved amino acids of the Exo I, Exo II and Exo III motifs which are characteristic of this class of exonucleases. Other conserved  
20 amino acids have been given a gray background. The three acidic amino acid side chains, i.e. two in the Exo I domain and one in the Exo III domain, are involved directly in binding the two Mg<sup>2+</sup> ions which participate in the enzymic hydrolysis. A third acidic amino acid side chain, which is located in the Exo II domain, binds one of the metal ions by way of hydrogen bond  
25 molecules. All these amino acid residues are highly conserved within the family and are also found in the human DAN according to the invention. The polypeptide according to the invention can therefore be described as being a poly(A)-specific 3'-exoribonuclease belonging to the RNaseD family.

30 The polypeptide according to the invention is prepared, for example, by expressing the nucleic acid according to the invention in a suitable expression system, as already described above, using methods which are well known to the skilled person. Examples of suitable host cells are the E.  
35 coli strains DH5, HB101 and BL21, the yeast strain *Saccharomyces cerevisiae*, the insect cell line Lepidopteran, e.g. from *Spodoptera Frugiperda*, or animal cells, such as COS, Vero, 293 and HeLa cells, all of which are available generally.

In particular, said parts of the polypeptide can also be synthesized by classical peptide synthesis (Merrifield technique). They are particularly suitable for obtaining antisera which can be used for screening suitable gene expression libraries for the purpose of gaining access to other functional variants of the polypeptide according to the invention.

The present invention therefore also relates to a process for preparing a polypeptide according to the invention, with a nucleic acid according to the invention being expressed in a suitable host cell and being isolated, where appropriate.

The present invention furthermore also relates to antibodies which react specifically with the polypeptide according to the invention, with the abovementioned parts of the polypeptide either themselves being immunogenic or being able to be made immunogenic, or to have their immunogenicity increased, by being coupled to suitable carriers, for example bovine serum albumin.

The antibodies are either polyclonal or monoclonal. Their preparation, which is also part of the subject-matter of the present invention, is effected, for example, in accordance with well known methods, by immunizing a mammal, for example a rabbit, with the polypeptide according to the invention or said parts thereof, where appropriate in the presence of, e.g., Freund's adjuvant and/or aluminum hydroxide gels (see, e.g., Diamond, B.A. et al. (1991) The New England Journal of Medicine, 324(13), 875-879). The polyclonal antibodies which have been produced in the animal due to an immunological reaction can then be readily isolated from the blood using well known methods and purified, for example, by way of column chromatography. Preference is given to purifying the antibodies by affinity chromatography, in which, for example, the C-terminal DAN fragment has been coupled to an NHS-activated HiTrap column.

Monoclonal antibodies can, for example, be prepared using the known method of Winter & Milstein (Winter, G. & Milstein, C. (1991) Nature, 349, 293).

The present invention furthermore also relates to a pharmaceutical which comprises a nucleic acid according to the invention or a polypeptide according to the invention and, where appropriate, suitable additives or

adjuvants, and to a process for producing a pharmaceutical for treating cancer, autoimmune diseases, in particular multiple sclerosis or rheumatoid arthritis, Alzheimer's disease, allergies, in particular neurodermatitis, type I allergies or type IV allergies, arthrosis, 5 atherosclerosis, osteoporosis, acute and chronic infectious diseases and/or diabetes, and/or for influencing the metabolism of the cell, in particular in association with immunosuppression, very particularly in association with transplantations, in which pharmaceutical a nucleic acid according to the invention, for example an antisense nucleic acid, or a polypeptide 10 according to the invention is formulated together with pharmaceutically acceptable additives and/or adjuvants.

A pharmaceutical which comprises the nucleic acid according to the invention in naked form or in the form of one of the above-described 15 vectors which are effective in gene therapy, or in the form in which it is complexed with liposomes, is very particularly suitable for gene therapy applications in humans.

Examples of suitable additives and/or adjuvants are a physiological sodium 20 chloride solution, stabilizers, proteinase inhibitors, nuclease inhibitors, etc.

The present invention furthermore also relates to a diagnostic agent which comprises a nucleic acid according to the invention, a polypeptide according to the invention or antibodies according to the invention and, 25 where appropriate, suitable additives and/or adjuvants, and to a process for preparing a diagnostic agent for diagnosing cancer, autoimmune diseases, in particular multiple sclerosis or rheumatoid arthritis, Alzheimer's disease, allergies, in particular neurodermatitis, type I allergies or type IV allergies, arthrosis, atherosclerosis, osteoporosis, acute and chronic 30 infectious diseases and/or diabetes, and/or for analyzing the metabolism of the cell, in particular the immune status, very particularly in association with transplantations, in which diagnostic agent suitable additives and/or adjuvants are added to a nucleic acid according to the invention, a polypeptide according to the invention or antibodies according to the 35 invention.

For example, according to the present invention, the nucleic acid according to the invention can be used to prepare a diagnostic agent which is based on the polymerase chain reaction (PCR diagnostics, e.g. as described in

EP-0200362) or on a Northern blot, as described in more detail in Example 5. These tests are based on the specific hybridization of the nucleic acid according to the invention with the complementary counterstrand, usually of the corresponding mRNA. In this connection, the  
5 nucleic acid according to the invention can also be modified, as described, for example, in EP 0063879. Preference is given to labeling a DNA fragment according to the invention with suitable reagents, for example radioactively with  $\alpha$ -P<sup>32</sup>-dATP or nonradioactively with biotin, using well-known methods, and incubating it with isolated RNA which has preferably  
10 been bonded to suitable membranes composed, for example, of cellulose or nylon. It is furthermore advantageous to fractionate the isolated RNA according to size, e.g. by means of agarose gel electrophoresis, before hybridization and binding to a membrane. In this way, when the quantity of RNA examined from each tissue sample is the same, it is then possible to  
15 determine the quantity of mRNA which has been labeled specifically by the probe.

Another diagnostic agent comprises the polypeptide according to the invention or the immunogenic parts thereof which have been described in  
20 more detail above. The polypeptide, or the parts thereof, which are preferably bound to a solid phase, e.g. composed of nitrocellulose or nylon, can, for example, be brought into contact in vitro with the body fluid, e.g. blood, to be investigated in order thereby to be able to react, for example, with autoimmune antibody. The antibody-peptide complex can then, for  
25 example, be detected using labeled anti-human IgG or anti-human IgM antibodies. The label is, for example, an enzyme, such as peroxidase, which catalyzes a color reaction. The presence of autoimmune antibodies, and the quantity of these antibodies which is present, can consequently be detected readily and rapidly by way of the color reaction.

30 Another diagnostic agent comprises the antibodies according to the invention themselves: These antibodies can be used, for example, to readily and rapidly investigate a human tissue sample to determine whether the polypeptide in question is present. In this case, the antibodies according to the invention are, for example, labeled with an enzyme as already described above. The specific antibody-peptide complex can thereby be detected readily and just as rapidly by way of an enzymic color reaction.

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The present invention also relates to a test for identifying functional interactors, such as inhibitors or stimulators, comprising a nucleic acid according to the invention, a polypeptide according to the invention or the antibodies according to the invention, and, where appropriate, suitable additives and/or adjuvants.

An example of a suitable test for identifying functional interactors is the so-called two-hybrid system (Fields, S. & Sternglanz, R. (1984) Trends in Genetics, 10, 286). In this test, a cell, for example a yeast cell, is transformed or transfected with one or more expression vectors which express a fusion protein which contains the polypeptide according to the invention and a DNA-binding domain from a known protein, for example from E. coli Gal4 or LexA, and/or express a fusion protein which contains an unknown polypeptide and a transcription-activating domain, for example from Gal4, herpes virus VP16 or B42. In addition, the cell contains a reporter gene, for example the E. coli LacZ gene, "green fluorescence protein" or the yeast amino acid biosynthesis genes His3 or Leu2, which is controlled by regulatory sequences such as the LexA promoter/operator or by a so-called upstream activation sequence (UAS) which is present in the yeast. The unknown polypeptide is, for example, encoded by a DNA fragment which is derived from a gene library, for example from a human gene library. Normally, the above-described expression vectors are used to prepare a cDNA gene library directly in yeast so that the test can be performed immediately thereafter.

For example, the nucleic acid according to the invention is cloned in a yeast expression vector in functional unity onto the nucleic acid encoding the LexA DNA-binding domain, such that the transformed yeast expresses a fusion protein composed of the polypeptide according to the invention and the LexA DNA-binding domain. In another yeast expression vector, cDNA fragments from a cDNA gene library are cloned in functional unity onto the nucleic acid encoding the Gal4 transcription-activating domain, such that the transformed yeast expresses a fusion protein composed of an unknown polypeptide and the Gal4 transcription-activating domain. The yeast which is transformed with the two expression vectors, and which is, for example, Leu2<sup>-</sup>, additionally contains a nucleic acid which encodes Leu2 and which is controlled by the LexA promoter/operator. If a functional interaction takes place between the polypeptide according to the invention and the unknown polypeptide, the Gal4 transcription-activating domain

then binds, by way of the LexA DNA-binding domain, to the LexA promoter/operator, resulting in the latter being activated and the Leu2 gene being expressed. This then enables the Leu2<sup>-</sup> yeast to grow on minimal medium which does not contain any leucine.

5

- When the LacZ reporter gene or the green fluorescence protein reporter gene is used instead of an amino acid biosynthesis gene, activation of transcription can be detected by blue- or green-fluorescent colonies being formed. The blue color or fluorescence color can then be readily quantified
- 10      in a spectrometer, for example at 585 nM in the case of a blue color.

- In this way, it is possible to screen gene expression libraries readily and rapidly for polypeptides which interact with the polypeptide according to the invention. The new polypeptides which have been found can then be
- 15      isolated and subjected to further characterization.

- Another possibility of applying the two-hybrid system is that of using other substances, such as chemical compounds, to influence the interaction between the polypeptide according to the invention and a known or
- 20      unknown polypeptide. In this way, it is also readily possible to find novel, valuable, chemically synthesizable active compounds which can be employed as therapeutic agents. The present invention is therefore not only directed toward a process for finding polypeptide-like interactors but also extends to a process for finding substances which are able to interact
- 25      with the above-described protein-protein complex. Within the meaning of the present invention, such peptide-like interactors, and also chemical interactors, are therefore designated functional interactors which are able to have an inhibitory or stimulatory effect.

- 30      Another possible application of the polypeptide according to the invention is the poly(A)-specific degradation of nucleic acids, in particular of mRNA. The poly(A)-specific degradation of nucleic acids can be of particular use in research laboratories.
- 35      The following figures and examples are intended to clarify the invention without limiting it.

Description of the figures and the most important sequences

SEQ 11 shows the amino acid sequence of the human DAN, containing domains for Exo I (ADFFAIDGEFSGIS), Exo II LVIGHNMLLDVMHTVH) and Exo III (SEQLHEAGYDAYITGLC).

SEQ 12 shows the nucleic acid sequence of the human DAN, including the start codon (pos. 58) and the stop codon (pos. 1977) and the subsequent 3'-UTR.

Fig. 1 shows the elution profile of human DAN on MonoQ (Fig. 3A) and SDS-PAGEs (Figs. 1B and 1C)

Fig. 2 shows a Western blot of recombinant human DAN and native bovine DAN

Fig. 3 shows the deadenylation of mRNA, the accumulation of deadenylated RNA and the influence of PABI

Fig. 4 shows a comparison of known nucleases with the human DAN according to the invention.

20  
**Examples**

1. Identification of human DAN cDNA clones

25 Bovine DAN was purified as follows and as described in Körner, G. & Wahle, E. (1997), see above:

All the steps of the purification were carried out at 4°C. Between the purification steps, the samples were frozen in liquid nitrogen and frozen at 30 -80°C. The following basic buffer was used: 50 mM Tris/HCl, 1 mM EDTA, 10% (v/v) glycerol, 1 mM dithiothreitol, 0.4 µg of leupeptin hemisulfate/ml, 0.7 µg of pepstatin/ml, 0.5 mM phenylmethylsulfonyl fluoride, 0.02% (v/v) Nonidet P-40, pH 7.9. Different concentrations of KCl were added to this basic buffer in the various purification steps, as described below.

35 Fresh calf thymus was obtained from a local slaughterhouse, transported on ice and stored at -80°. One kg was thawed in 2 l of basic buffer containing 50 mM KCl and homogenized in a Waring Blender homogenizer, initially at low speed, then at medium speed and finally at the

highest speed. The homogenate was centrifuged at  $16\ 000 \times g$  for 1 h and the supernatant was decanted through a wide-mesh gauze (Wahle, E., J. Biol. Chem., 266, 1991).

- 5 This calf thymus extract was loaded onto a DEAE-sepharose FF column (column volume 4 l) and eluted from the column at a flow rate of 3 l/h using a salt gradient of from 50 to 600 mM KCl in a volume which was 2.5 times that of the column. Active fractions were eluted from the column at a salt concentration of between 75 and 200 mM KCl. The fractions were  
10 collected, combined, treated with ammonium sulfate to give a 30% saturated solution, and stirred on ice for 1.5 h. After a centrifugation (10 800  $\times g$  for 30 min, applies to all the centrifugation steps mentioned below), the supernatant was adjusted to 50% saturation with ammonium sulfate and was once again stirred on ice and centrifuged. The sediment  
15 was resuspended in 400 ml of basic buffer containing 50 mM KCl and dialyzed against 2  $\times$  4.5 l of basic buffer for 10 h; it was then centrifuged once again. A 1.4 l sepharose-blue column (7  $\times$  36 cm) was equilibrated with basic buffer containing 50 mM KCl and the dialyzed extract was loaded onto the column. The column was washed with 1.5 bed volumes of  
20 basic buffer containing 250 mM KCl and eluted with one bed volume of basic buffer containing 1 M KCl (flow rate 2 l/h).

The active fractions from two preparations from in each case 1.2 kg of calf thymus were combined and precipitated with ammonium sulfate (60%  
25 saturation). After the centrifugation, the sediment was taken up in 200 ml of basic buffer containing 50 mM KCl, dialyzed for 12 h against 3  $\times$  4 l of the same buffer and loaded, in two portions, onto a heparin-sepharose column (2.5  $\times$  37 cm). The column were washed with 1.5 bed volumes of basic buffer containing 50 mM KCl and then eluted with 10 bed volumes in a  
30 gradient of up to 500 mM KCl (flow rate: 145 ml/h). The DAN activity eluted between 80 and 150 mM KCl. The active fractions were combined and dialyzed for 4 h against basic buffer containing 30 mM KCl. The dialysate was centrifuged and chromatographed in two portions on a MonoQ FPLC column (bed volume 8 ml). The column was washed with two bed volumes  
35 of basic buffer containing 50 mM KCl and then eluted from the column with a 320 ml gradient containing an increasing concentration of salt (final concentration: 500 mM KCl, flow rate: 2.5 ml/h). The DAN activity eluted at approx. 160 mM KCl. The active fractions (40 ml) were combined and dialyzed for 4 h against 2 l of basic buffer containing 30 mM KCl,

- centrifuged and loaded onto a further MonoQ column (1 ml bed volume, flow rate: 0.9 ml/h). The DAN activity bound to the column was eluted with basic buffer containing 500 mM KCl and loaded, in four portions, onto a Superdex HR 10/30 FPLC column (equilibrated with basic buffer containing 5 300 mM KCl, flow rate: 0.15 ml/h) (Körner, Ch. G. & Wahle, E. (1997), see above).

Different fractions which were eluted from a Superdex column, and which contained the DAN activity, were collected and fractionated through a 10 Phenyl Superose column (Körner, G. & Wahle, E. (1997), see above). The active fractions were collected and dialyzed against buffer (50 mM Tris HCl, pH 7.9, 1 mM EDTA, 10% (v/v) glycerol, 1 mM dithiothreitol, 0.4 mg of leupeptin hemisulfate/ml, 0.7 mg of pepstatin/ml, 0.5 mM phenylmethylsulfonate (PMSF), 0.02% (v/v) Nonidet p-40, 50 mM KCl). 15 After having been centrifuged, the dialysate was loaded onto a 100 ml Smart MonoQ (PC 1.6/5) column. The column was then washed with 200 ml of buffer. The bound proteins were eluted in a gradient containing an increasing concentration of salt (final concentration 500 mM KCl) and having a volume equal to 40 column bed volumes. The fractions which 20 were positive for DAN activity eluted at a KCl concentration of approx. 200 mM KCl. The two fractions having the highest DAN activity were analyzed by SDS polyacrylamide gel electrophoresis. This was then followed by in-situ hydrolysis with the protease Lys-C, fractionation by HPLC and sequencing of the peptides. The following sequences were 25 found:

1. KSFNFYVFPK,
2. KPFNRSSPD(V/K)K,
3. KYAESYWIQTYADYVG and also
4. a mixture composed of: KEQEELNDA and KLFLMRVMD.

30 It was not possible to determine the N-terminal sequence of the purified bovine DAN.

Subsequently, EST (expressed sequence tags) database searches were carried out using the BLAST program (NCBI). The following clones were 35 identified:  
I.M.A.G.E. Consortium clone ID 645295 through peptides 1 and 2, and I.M.A.G.E. Consortium clone ID 301901 through peptide 3.

The clones which had been found were sequenced completely on an AB1373A sequencing appliance using the ABI Dye Terminator Cycle sequencing kit.

- 5 In this connection, it was found that the I.M.A.G.E. consortium clone ID 301901 encodes the 176 C-terminal amino acids of DAN and the entire 3'-UTR, whereas I.M.A.G.E. consortium clone ID 645295 encodes the entire ORF and the 5' UTR and a part of the 3'-UTR, corresponding to a protein composed of 639 amino acids and having a molecular mass of 73.5 kDa  
10 10. The 57 nucleotides which are located upstream of the first AUG codon do not contain any stop codon in the reading frame. The sequences (AGAAUUGG) surrounding this AUG codon conform to the so-called Kozak rules (Kozak, 1991) which describe preferred starting sequences for the translation start. A 0.7 kB 3'-UTR is present in cDNA clone 645 295 and a  
15 15. poly(A) tail is present at the end of cDNA clone 301901. The poly(A) tail is preceded upstream by the rare polyadenylation signal AUUAAA (SEQ 12).

## 2. Preparing expression vectors

- 20 The plasmid pGMMCS was constructed in the following manner:

The T7 expression vector pGM10 (Martin, 1996), which contained the PABII cDNA sequence (Nemeth, 1996) having an N-terminal Met-Ala-His6 tag, was digested with Xho I and BamH I and the fragment, which  
25 25. contained the 3' moiety of the PAB II, was replaced with a Xho I/BamH I fragment from the multiple cloning site of the pBluescript KS (+/-) plasmid. The resulting plasmid contains a sequence which is regulated by the T7 promoter and begins with Met-Ala His6, followed by the 5' moiety of PAB II and a multiple cloning site. An NDE I cleavage site is located between the  
30 30. His6 tag and the PAB II sequence and can be used, together with the multiple cloning site, to replace the remaining PAB II sequence with any optional coding sequence.

The plasmid pGMMCS301901 was prepared in the following manner:

- 35 A part of the sequence of the human DAN clone containing the 176 C-terminal amino acids was amplified from clone 301901 by PCR using the following conditions:  
15 cycles: 45 s at 94°C, 45 s at 54°C and 3.5 min at 72°C. Pfu polymerase and the primers Nde I 301901:

CCATATCCATATGCTTTCAGTGCCTTCCTAAC and Xho I 301901: AGTACTCGAGTTACAATGTGTCAGG. Following digestion with Nde I and Xho I, the resulting cDNA fragments were purified using the Qia-Ex kit (Qiagen GmbH, Hilden) and integrated into the Nde I- and Xho I-digested pGMMCS vector. The sequence was confirmed by sequencing.

The plasmid pGMMCS645295 was prepared as follows:

The coding sequence of the human DAN clone was amplified from clone  
10 645295 by PCR using the following conditions:  
20 cycles: 45 s at 94°C, 45 s at 54°C and 3.5 min at 72°C. Pfu polymerase  
and the primers Nde I 645295:  
AGTGTGCGATATGGAGATAATCAGGAGCA and Xho I 645295:  
AGTACTCAGCGGTTGCTGCCCTCA. The resulting product was cloned  
15 into the vector pCR2.1 using the TA cloning kit (InVitrogen®). Following  
digestion with Nde I and Xho I, the resulting cDNA fragments were purified  
using the Qia-Ex kit (Qiagen) and integrated into the Nde I- and Xho I-  
digested pGMMCS vector. The major part of the PCR-generated sequence  
was then removed by digesting with Dra III and BstE II and replaced with  
20 the corresponding fragment of the original clone. The new sequence was  
confirmed by sequencing.

### 3. Preparing antibodies

25 Antibodies directed against the C-terminal moiety of human DAN were produced as follows:

The plasmid pGMMCS301901 was transformed into BL21 (pLysC). The cells were incubated at 37°C in SOB medium containing 200 mg of carbenicillin ml up to an OD<sub>600</sub> of 1.9. 200 mM isopropyl-β-D-thiogalactoside was then added and the mixture was incubated for a further 5 h. The cells were harvested and taken up in buffer A (100 mM NaH<sub>2</sub>PO<sub>4</sub>, 10 mM Tris, 8M urea, pH 7.9). The cells were lysed by ultrasonication and the lysate was centrifuged and incubated with 3 ml of Ni-NTA column material at room temperature for 2 h. The column material was packed into a column and washed with 70 ml of buffer A, pH 6.3. It was then washed with 15 ml of buffer B (300 mM NaCl, 10% (v/v) glycerol, 50 mM Tris, 0.01% (v/v) Nonidet-P40, 8M urea, pH 7.9). The protein was refolded on the column by decreasing the urea content with 2 gradients (45 ml/h, gradient 1: buffer B containing from 8 to 4 M urea, at room

temperature, gradient II: containing from 4 to 0 M urea, at 4°C). the protein was eluted from the column with buffer B which contained 500 mM imidazole, dialyzed against buffer B which does not contain any urea and then used for immunizing rabbits.

5

Anti-DAN antibodies were affinity-purified as follows: the C-terminal DAN fragment was coupled to an NHS-activated HiTrap column (1 ml volume, Pharmacia, Freiburg) in accordance with the manufacturer's instructions. 3 ml of serum were loaded onto the column and eluted in accordance with 10 the manufacturer's instructions. 300 mg of BSA/ml and 0.02% (w/v) NaN<sub>3</sub> were added to the fractions and the buffer was replaced by dialysis.

#### 4. Expressing and purifying human DAN

15 Human DAN was expressed in E. coli as a fusion protein having an N-terminal His tag (Met-Ala-His<sub>6</sub> tag) in the following manner:

The plasmid pGMMCS645295 was transformed into BL21 (pLysC). The 20 cells were cultured, at 37°C, in 50 ml of LB medium containing 100 mg of carbenicillin/ml and 24 mg of chloramphenicol/ml and then transferred into a 500 ml culture without chloramphenicol and subjected to further incubation at 33°C. After an OD<sub>600</sub> at 1.3 had been reached, 100 mM isopropyl-β-D-thiogalactoside was added and the culture was incubated for a further hour. The cells were harvested and taken up in buffer A (50 mM 25 Tris, 300 mM KCl, 0.1 mM MgAc, 1 mM β-mercaptoethanol, 0.4 mg of leupeptin/ml, 0.7 mg of pepstatin/ml, 0.5 mM PMSF, pH 7.9). The cells were lysed by ultrasonification and the lysate was centrifuged and incubated, at 4°C for 2 h, with 2 ml of Ni<sup>2+</sup>-NTA column material. The column material was packed into a column and washed with 25 ml of buffer 30 A and then with 20 ml of buffer B (buffer A and 10% (v/v) glycerol, 0.02% (v/v) nonidet-P40, without magnesium, pH 6.3). The protein was then eluted with 5 ml of buffer C (buffer B containing 500 mM imidazole). There then followed a dialysis against buffer D (50 mM Tris, 20 mM KCl, 1 mM EDTA, 5 mM PMSF, 10% (v/v) glycerol, 0.02 (v/v) Nonidet-P40, pH 7.9). 35 The preparation was centrifuged and loaded onto a MonoQ column (1 ml bed volume). The column was then washed with 5 bed volumes of buffer containing 50 mM KCl and eluted with a gradient having a volume equal to 40 bed volumes and a final concentration of 500 mM KCl. The DAN activity eluted in a sharp peak at approx. 190 mM KCl (Fig. 1A).

The purification resulted in a virtually homogeneous preparation of a protein having the expected molecular mass (Figs. 1B and 1C). The protein exhibits a 3'-exoribonuclease activity which is specific for poly(A) (see Example 6). It follows from this that the found cDNA clone encodes a  
5 human DAN.

### 5. Northern blot analysis

RNA was isolated from HeLa cells using a method described by  
10 Chomczynski, P. & Sacchi, N. (1987) Anal. Biochem. 162, 156. Poly(A)  
mRNA was purified using oligo(dT) cellulose in accordance with a method  
described by Sambrook, J. et al. (1989) Molecular Cloning. A Laboratory  
Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring  
Harbor, N.Y. The resulting RNA was fractionated on a 1% agarose  
15 formaldehyde gel, transferred by capillary blotting to a Hybond N+  
membrane (from Amersham Buchler, Brunswick) and fixed on the  
membrane by incubating at 80°C for 2 h. The sequence encoding human  
DAN was amplified by PCR, labeled with  $\alpha$ -<sup>32</sup>P-dATP by random priming  
and used as a probe. The hybridization was carried out as described  
20 (Ausubel) and the membrane was washed under high stringency. The  
Northern blot analysis using HeLa cell poly(A)<sup>+</sup> RNA showed one single  
3.1 kB fragment which hybridized with the DAN probe. This size agrees  
well with the size of the cDNA clone.

### 25 6. DAN activity test

The assay for DAN activity was carried out as described (Körner, Chr G. &  
Wahle, E. (1997), see above). SDS polyacrylamide gel electrophoresis  
30 (SDS-PAGE) was carried out as described by Laemmli, U.K. (1970) Nature  
227, 680.

Like the bovine enzyme, the recombinant DAN is active under two different  
reaction conditions depending on the neutralization of phosphate charges.  
In the absence of salt, its activity is completely dependent on the presence  
35 of spermidine with the optimum concentration being 1 mM. Under these  
conditions, the specific activity of the recombinant DAN (79 000 U/mg) is  
not significantly different from the activity of purified bovine DAN  
(110 000 U/mg). In the presence of spermidine, the activity of DAN is  
inhibited by salt. In the absence of spermidine, the activity of the enzyme is

dependent on salt, with the concentration optimum being 150 mM potassium acetate. Bovine DAN behaves in a similar manner under these conditions as well. However, the specific activity of the recombinant protein (960 U/mg) is lower under these conditions than is that of the enzyme  
5 purified from calf thymus (8070 U/mg). This difference can be explained either by a post-translational modification or by contaminating proteins being present in the preparation of the bovine enzyme.

When a capped polyadenylated RNA was incubated with the DAN  
10 preparations in the presence of salt, the poly(A) tail was degraded and completely deadenylated RNA accumulated transiently (Fig. 3). If the assay was carried out in the presence of PABI, the activity was partially inhibited and step-wise degradation of the poly(A) tail was observed. The length of the predominantly degradation products differed by approx. 30  
15 nucleotides, a phenomenon which was evidently due to the PABI binding. These results concur with the investigations carried out on bovine protein (Körner, Chr. & Wahle, E. (1997), see above). Taken together, these results demonstrate that the recombinant protein is a poly(A)-specific 3'-exoribonuclease.  
20

#### 7. Western blot analyses

Western blot analyses were carried out as follows: the proteins were fractionated by SDS-PAGE and transferred to a nitrocellulose membrane  
25 using the semidry method (Kyse-Andersen, 1984). The blots were incubated in TNT buffer (20 mM Tris-HCl, 150 mM NaCl, 0.05% (v/v) Tween 20, pH 7.5) containing 5% (w/v) dried skimmed milk. The same buffer was used for the incubation with antiserum and the washing steps. The blots were incubated with the antibodies at room temperature for  
30 2 - 3 h and then washed. The bound antibodies were detected using a peroxidase-conjugated pig anti-rabbit antibody (DAKO, Glostrup, Denmark) and chemiluminescence staining (SuperSignal kit, Pierce).

The analyses demonstrate that rabbit antibodies which were generated  
35 against a C-terminal fragment of DAN are able to precipitate DAN from partially purified fractions. The antibodies recognize both bovine and human DAN in a Western blot (Fig. 4). In this experiment, the recombinant DAN appears to somewhat larger than the enzyme in SDS lysates obtained from HeLa cells, something which can be explained by the

introduction of the tag (1004 Da) into the sequence. If the first AUG in the cDNA sequence had not been the start codon which was used, the natural DAN from HeLa cells would have had to have been at least 1260 Da larger than the tagged recombinant protein. These results, too, confirm that the  
5 first AUG sequence in the cDNA clone is used as the start codon in vivo.

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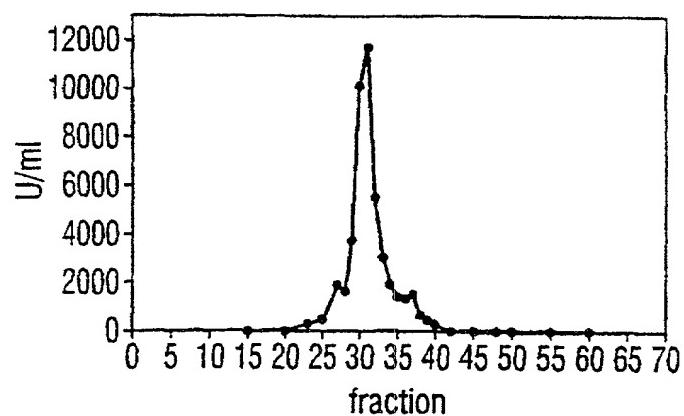
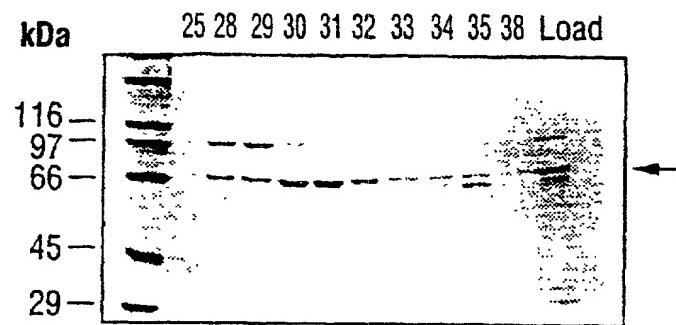
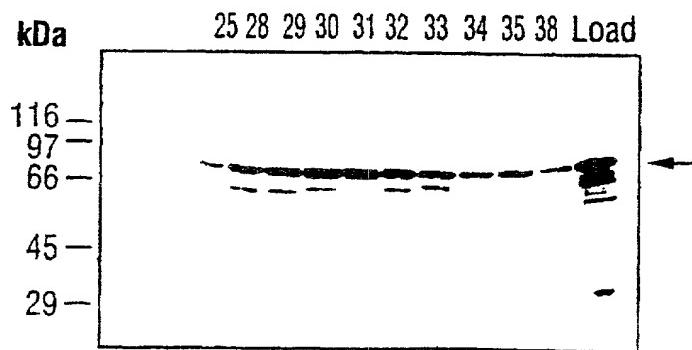
## Patent claims

1. A nucleic acid which encodes a human deadenylating nuclelease (DAN) having an amino acid sequence as depicted in SEQ 11 or a functional variant thereof, and parts thereof having at least 8 nucleotides, with SEQ 11 being part of the claim.
  2. A nucleic acid as claimed in claim 1, wherein the nucleic acid is a DNA or RNA, preferably a double-stranded DNA.
  3. A nucleic acid as claimed in claim 1 or 2, wherein the nucleic acid is a DNA having a nucleic acid sequence as depicted in SEQ 12 from position 58 to 1977, with SEQ 12 being part of the claim.
  4. A nucleic acid as claimed in claim 3, wherein the nucleic acid contains one or more noncoding sequences and/or a polyA sequence.
  5. A nucleic acid as claimed in one of claims 1-4, wherein the nucleic acid is contained in a vector, preferably in an expression vector or a vector which is effective in gene therapy.
  6. A process for preparing a nucleic acid as claimed in one of claims 1-4, wherein the nucleic acid is synthesized chemically or isolated from a gene library using a probe.
  7. A polypeptide having an amino acid sequence as depicted in SEQ 11 or a functional variant thereof, and parts thereof having at least 6 amino acids.
  8. A process for preparing a polypeptide as claimed in claim 7, wherein a nucleic acid as claimed in one of claims 1-5 is expressed in a suitable host cell.
  9. An antibody directed against a polypeptide as claimed in claim 7.

10. A process for preparing an antibody as claimed in claim 9, wherein a mammal is immunized with a polypeptide as claimed in claim 7 and the resulting antibodies are isolated, where appropriate.
- 5 11. A pharmaceutical which comprises a nucleic acid as claimed in one of claims 1-5 or a polypeptide as claimed in claim 7 and, where appropriate, pharmaceutically acceptable additives and/or adjuvants.
- 10 12. A process for producing a pharmaceutical for treating cancer, autoimmune diseases, in particular multiple sclerosis or rheumatoid arthritis, Alzheimer's disease, allergies, in particular neurodermatitis, type I allergies or type IV allergies, arthrosis, atherosclerosis, osteoporosis, acute and chronic infectious diseases and/or diabetes, and/or for influencing the metabolism of the cell, in particular in association with immunosuppression, very particularly in association with transplantations, wherein a nucleic acid as claimed in one of claims 1-5 or a polypeptide as claimed in claim 7 or antibodies as claimed in claim 9 is formulated together with a pharmaceutically acceptable additive and/or adjuvant.
- 15 13. A diagnostic agent which comprises a nucleic acid as claimed in one or claims 1-5 or a polypeptide as claimed in claim 7 or antibodies as claimed in claim 9 and, where appropriate, suitable additives and/or adjuvants.
- 20 14. A process for preparing a diagnostic agent for diagnosing cancer, autoimmune diseases, in particular multiple sclerosis or rheumatoid arthritis, Alzheimer's disease, allergies, in particular neurodermatitis, type I allergies or type IV allergies, arthrosis, atherosclerosis, osteoporosis, acute and chronic infectious diseases and/or diabetes, and/or for analyzing the metabolism of the cell, in particular the immune status, very particularly in association with transplantations, wherein a pharmaceutically acceptable excipient is added to a nucleic acid as claimed in one of claims 1-5 or a polypeptide as claimed in claim 7 or antibodies as claimed in claim 9.
- 25
- 30
- 35

15. A test for identifying functional interactors, comprising a nucleic acid as claimed in one of claims 1-5 or a polypeptide as claimed in claim 7 or antibodies as claimed in claim 9 and, where appropriate, suitable additives and/or adjuvants.
- 5
16. The use of a nucleic acid as claimed in one of claims 1-5 or a polypeptide as claimed in claim 7 for identifying functional interactors.
- 10 17. The use of a nucleic acid as claimed in one of claims 1-5 for finding variants of human DAN, wherein a gene library is screened with said nucleic acid and the variant which is found is isolated.
- 15 18. The use of a polypeptide as claimed in claim 7 for the poly(A)-specific degradation of nucleic acids, in particular of mRNA.

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*Fig. 1A**Fig. 1B**Fig. 1C*

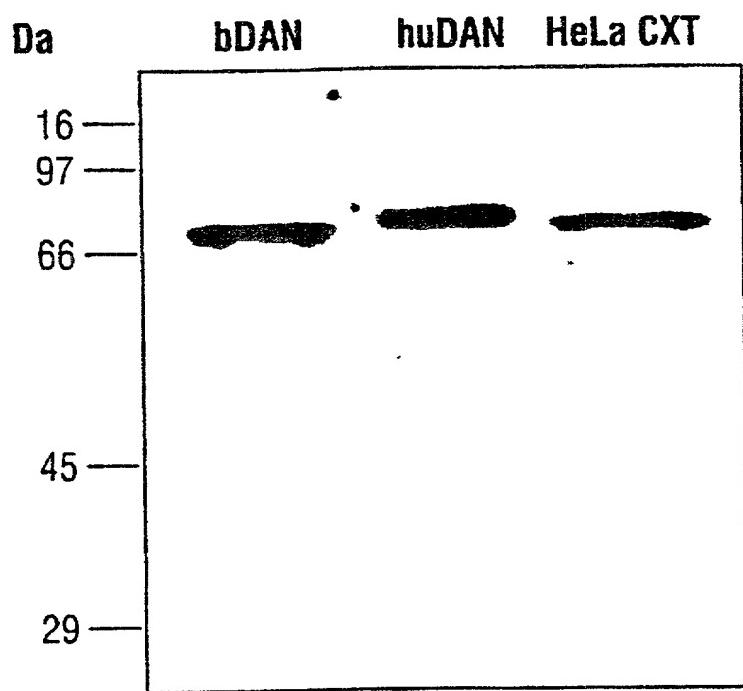
**Fig. 2**

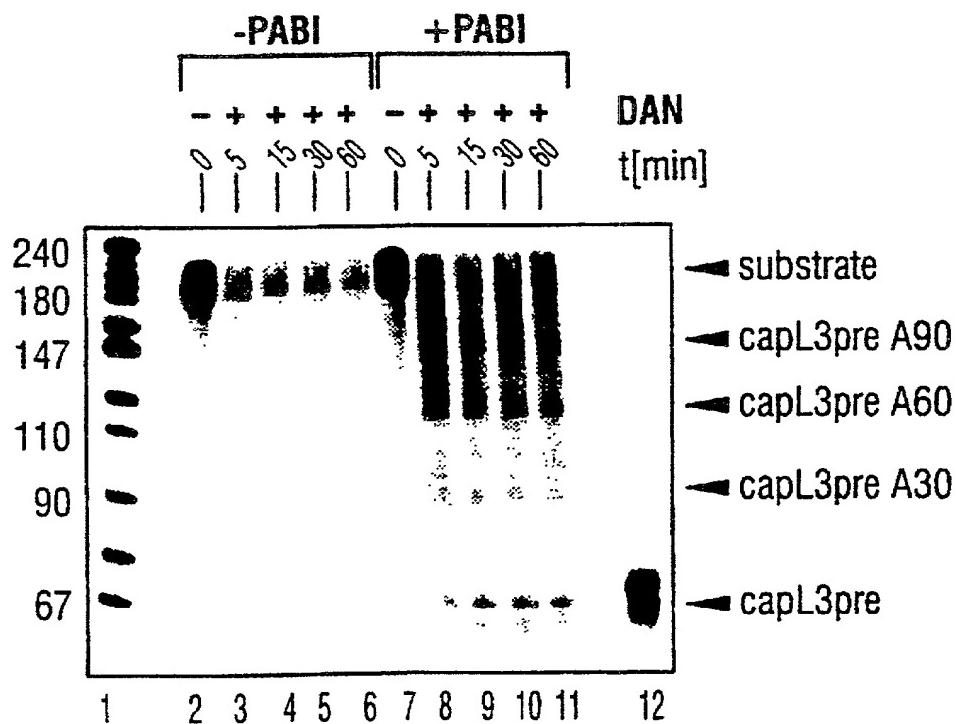
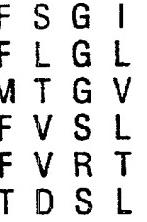
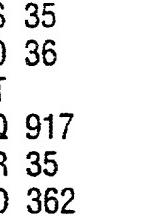
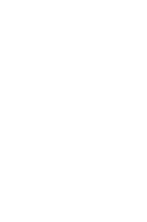
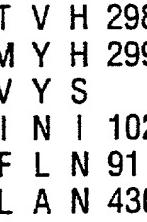
Fig. 3

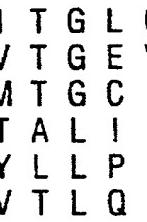
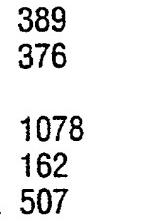
Fig. 4

huDAN	22 A D F F A I		35
K10C81	23 C D F V A I		36
A. thaliana	A D F V A I		
PAN2	904 G T L V A I		917
RNaseD	22 F P A I A L		35
EcoDNAPol1	349 A P V F A F		362

↑      ↑  
ExoI

huDAN	283 L V I G H N M L L		298
K10C81	284 L V V G H N S L L		299
A. thaliana	L I V G H N C F L		
PAN2	1011 V F V G H G L N N		1026
RNaseD	76 T K F L H A G S E		91
EcoDNAPol1	415 L K V G Q N L K Y		430

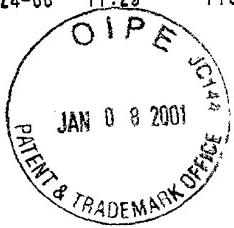
↑  
ExoII

huDAN	373 S E Q L		389
K10C81	370 E N V Y		376
A. thaliana	A G G K		
PAN2	1062 Q E G N		1078
RNaseD	146 E R Q E W Y		162
EcoDNAPol1	493 E E A G R		507

↑      ↑  
ExoIII

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10-24-00 11:29 From-CLARK AND ELBING



6174287045

T-271 P 02/04 F-392

PATENT  
ATTORNEY DOCKET NO: 50186/003001

## COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled HUMAN DEADENYLATING NUCLEASE, ITS PRODUCTION AND ITS USE, the specification of which

- is attached hereto.
- was filed on October 25, 2000 as Application Serial No. \_\_\_\_\_
- and was amended on \_\_\_\_\_
- was described and claimed in PCT International Application No. \_\_\_\_\_
- filed on \_\_\_\_\_ and as amended under PCT Article 19 on \_\_\_\_\_

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56(a).

**FOREIGN PRIORITY RIGHTS:** I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Country	Serial Number	Filing Date	Priority Claimed?
PCT	PCT/EP99/03071	May 5, 1999	Yes
Germany	DE 198 22 122.3	May 8, 1998	Yes

**PROVISIONAL PRIORITY RIGHTS:** I hereby claim priority benefits under Title 35, United States Code, §119(e) and §120 of any United States provisional patent application(s) listed below filed by an inventor or inventors on the same subject matter as the present application and having a filing date before that of the application(s) of which priority is claimed:

Serial Number	Filing Date	Status



## COMBINED DECLARATION AND POWER OF ATTORNEY

**NON-PROVISIONAL PRIORITY RIGHTS:** I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose all information I know to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56(a) which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Serial Number	Filing Date	Status

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: Paul T. Clark, Reg. No. 30,162, Karen L. Elbing, Ph.D. Reg. No. 35,238, Kristina Bieker-Brady, Ph.D. Reg. No. 39,109, Susan M. Michaud, Ph.D. Reg. No. 42,885, Mary Rose Scozzafava, Ph.D., Reg. No. 36,268, James D. DeCamp, Ph.D., Reg. No. 43,580, Sean J. Edman, Reg. No. 42,506, Timothy J. Douros, Reg. No. 41,716.

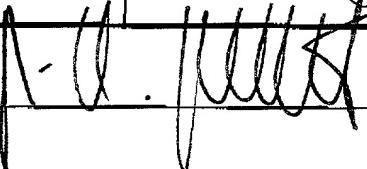
Address all telephone calls to: Karen L. Elbing, Ph.D. at 617/428-0200.

Address all correspondence to: Karen L. Elbing, Ph.D. at Clark & Elbing LLP, 176 Federal Street, Boston, MA 02110.

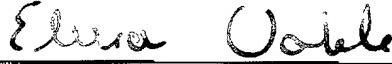
I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Full Name (First, Middle, Last)	Residence Address (City, State, Country)	Post Office Address (Street, City, State, Country)	Citizenship
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Signature: <i>Christoph Hüls</i>			Date: <i>16.11.00</i>

## COMBINED DECLARATION AND POWER OF ATTORNEY

Full Name (First, Middle, Last)	Residence Address (City, State, Country)	Post Office Address (Street, City, State, Country)	Citizenship
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Signature:			Date: <u>04/11/00</u>

Full Name (First, Middle, Last)	Residence Address (City, State, Country)	Post Office Address (Street, City, State, Country)	Citizenship
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Signature:			Date: <u>22/11/00</u>

Full Name (First, Middle, Last)	Residence Address (City, State, Country)	Post Office Address (Street, City, State, Country)	Citizenship
<u>Elmar Wahle</u>	<u>Wattenberg, Germany</u>	<u>An der Fels 20, D-35435, Wattenberg, Germany</u>	Germany
Signature:			Date: <u>3/12/00</u>

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

## (i) APPLICANT:

- (A) NAME: Aventis Research & Technologies GmbH & Co KG
- (B) STREET:
- (C) CITY: 65926 Frankfurt
- (D) FEDERAL STATE:
- (E) COUNTRY: Germany
- (F) POSTAL CODE: 65926

## (ii) TITLE OF INVENTION: Human deadenylating nuclease, its preparation and use

## (iii) NUMBER OF SEQUENCES: 29

## (iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: WINDOWS 3.1

## (2) INFORMATION FOR SEQ ID NO: 1

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10
- (B) TYPE: Amino acid sequence
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

## (ii) MOLECULE TYPE: Peptide

## (v) FRAGMENT TYPE: Internal fragment

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1

(2) INFORMATION FOR SEQ ID NO: 2

- (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 11
    - (B) TYPE: Amino acid sequence
    - (C) STRANDEDNESS: Single
    - (D) TOPOLOGY: Linear
  - (ii) MOLECULE TYPE: Peptide
  - (v) FRAGMENT TYPE: Internal fragment
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2

KPNRSPDV K

11

(2) INFORMATION FOR SEQ ID NO: 3

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    - (B) TYPE: Amino acid sequence
    - (C) STRANDEDNESS: Single
    - (D) TOPOLOGY: Linear
  - (ii) MOLECULE TYPE: Peptide
  - (v) FRAGMENT TYPE: Internal fragment
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3

KPNRSPDK K

11

(2) INFORMATION FOR SEQ ID NO: 4

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    - (C) STRANDEDNESS: Single
    - (D) TOPOLOGY: Linear

- (ii) MOLECULE TYPE: Peptide
  - (v) FRAGMENT TYPE: Internal fragment
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4

KYAESYWIQT YADYVG

16

(2) INFORMATION FOR SEQ ID NO: 5

- (i) SEQUENCE CHARACTERISTICS:
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    - (B) TYPE: Amino acid sequence
    - (C) STRANDEDNESS: Single
    - (D) TOPOLOGY: Linear
  - (ii) MOLECULE TYPE: Peptide
  - (v) FRAGMENT TYPE: Internal fragment
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5

KEQEEELNDA

8

(2) INFORMATION FOR SEQ ID NO: 6

- (i) SEQUENCE CHARACTERISTICS:
    - (A) LENGTH: 9
    - (B) TYPE: Amino acid sequence
    - (C) STRANDEDNESS: Single
    - (D) TOPOLOGY: Linear
  - (ii) MOLECULE TYPE: Peptide
  - (v) FRAGMENT TYPE: Internal fragment
  - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6

## (2) INFORMATION FOR SEQ ID NO: 7

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- (A) LENGTH: 34
- (B) TYPE: Nucleotide sequence
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

## (ii) MOLECULE TYPE: Miscellaneous nucleic acid: Primer DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7

CCATATCCAT ATGCTTTCA GTGCCTTC TAAC 34

## (2) INFORMATION FOR SEQ ID NO: 8

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: Nucleotide sequence
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

## (ii) MOLECULE TYPE: Miscellaneous nucleic acid: Primer DNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8

AGTACTCGAG TTACAATGTG TCAGG 25

## (2) INFORMATION FOR SEQ ID NO: 9

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29
- (B) TYPE: Nucleotide sequence
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

## (ii) MOLECULE TYPE: Miscellaneous nucleic acid: Primer DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9

AGTGTCGCAT ATGGAGATAA TCAGGAGCA

29

(2) INFORMATION FOR SEQ ID NO: 10

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 25
- (B) TYPE: Nucleotide sequence
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Miscellaneous nucleic acid: Primer DNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10

AGTACTCAGC GGTTTGCTGC CCTCA

25

(2) INFORMATION FOR SEQ ID NO: 11

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 639
- (B) TYPE: Amino acid sequence
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Protein

(v) FRAGMENT TYPE: N Terminus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11

09374062 0010804

MEIIRSNSFKS	NLHKVYQALE	EADFFAIDGE	FSGISDGPSV	SALTNGFDTP	EERYOKLKKH	60
SMDFLLFQFG	LCTFKYDYTD	SKYITKSFNF	XVFVKKPFNRS	SPDVKFVCQG	SSIDFLASQG	120
FDFPNKVFRNG	IPYLNQEER	QLREQYDEKR	SQANGAGALS	YVSPNTSKCP	VTIPEDQKXP	180
IDQVVEKIED	LLQSEENKNL	DLEPCCTGFQR	KLIYQTLSWK	YPKGIVHVEL	ETERKERRYIV	240
ISKVDEEERK	RREQQKHAK	QEELNDAVGF	SRVIHAIANS	GKLVIGHNML	LDVMHTVHQF	300
YCPLPADLSE	FKEMTTCVFP	RLLDTKLMAS	TQPFKDIINN	TSLAELEKRL	KETPFNPPKV	360
ESAEQGFSYD	TASEQLHEAG	YDAYITGLCF	ISMANYLGSF	LSPPPKIHVSA	RSKLIEPFFN	420
KLFLMRVMIDI	PYLNLEGPDL	QPKRDHVHLV	TFPKEWKTS	LYQLFSAFGN	IQISWIDDTS	480
AFVSLSQPEQ	VKIAVNNTSKY	AESYRIQTYA	EYMRKQEEK	QIKRKWTEDS	WKEADSKRLN	540
POCIFYTLQN	HYIRNNNSFTA	PSTVGKRNL	PSQEEAGLED	GVSGEISDTE	LEQTDSCREP	600
LSEGRRKKAKX	LKRMKKELSP	AGSISKNSPA	TLFEVPTDW			639

## (2) INFORMATION FOR SEQ ID NO: 12

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2941
- (B) TYPE: Nucleotide sequence
- (C) STRANDEDNESS: Double
- (D) TOPOLOGY: Linear

## (ii) MOLECULE TYPE: cDNA for mRNA

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12

GAACCGCTGA	GGCGGGCGCG	GGCCCGGGTG	GGGCCAAGGT	TCCGGCCACT	CTGCAGAAATG	60
GAGATAATCA	GGAGCAATT	TAAGAGTAT	CTTCACAAAG	TGTACCAAGGC	CATAGAGGAG	120
GCCGACTCT	TCGCCATCGA	TCGGGAGTT	TCAGGAATCA	GTGATGGACC	TTCACTCTCT	180
GCATTAACAA	ATGGTTTG	CACTCCGAA	GAGAGTATC	AGAACGTTAA	AAAGCATTC	240
ATGGACTTTT	TGCTATTCA	GTTCGGCCTT	TGCACTTTTA	AGATATGACTA	CACAGATTCA	300
AACTATATAA	CGAAGTCATT	TAACCTCTAT	GTTCCTCCGA	AACCCCTCAA	TAGATCCTCA	360
CCAGATGTCA	AATTGTTTG	TCAGAGCTCC	AGCATATGAT	TTCTAGGAG	CCACCGATT	420
GATTTTATAA	AAGTTTTTCG	AAATGGAATT	CCATATTAA	ATCAGGAARGA	AGAAAGACAG	480
TTAACGAGC	AGTATGATGA	AAAAGCTCA	CAGCGGAATG	GTGCAGGAGC	TCTGTCCTAT	540
GTATCTCTA	ACACTTCAA	ATGTCTGTC	ACGATTCCTG	AGGATCAAA	GAAGTTTATT	600
GACCAAGTGG	TAGAGAAAAT	AGAGGATTAA	TTACRAAGTG	AGAAAACAA	GAACCTGGAT	660
TTAGAGCCAT	GTACCGGGTT	CCAAAGAAA	CTAATTATC	AGACTTTGAG	CTGGAACTAT	720
CCGAAAGGCA	TTCATGTTGA	GACTTTAGAA	ACTGAAAAGA	AGGAGCGATA	TATAGTTATC	780
AGCAAAGTAG	ATGAAAGAAGA	ACGCAAAGA	AGAGCAGCAG	AGAAACATGC	CAAAGAACAG	840
GAGGAGCTGA	ATGATGCTGT	GGGATTCTCT	AGAGTCATT	ACGCCATTGC	TAATCCGG	900
AAACTTGTAA	TTGGACACAA	TATGCTTTG	GACGTATGC	ACACAGTTCA	TCAGTTCTAC	960
TGCCCTCTGC	CTGGGACTT	AAGTGAGTT	AAAGAGATGA	CAACATGTGT	TTTCCCCAGA	1020
CTCTTGATA	CTAAATTGAT	GGCCAGCACA	CAACCTTTA	AGGATATCAT	TAACAAACAA	1080
TCCCCTGGCG	AATTGGAAA	GGGGTTAAAA	GAGACACCTT	TCAACCCCTCC	TAAGTTGAA	1140
ACTGCCGAAG	TTTTTCCAAG	TTATGACACA	GCCTCTGAAAC	ARCTCCACGA	GGCAGGCTAC	1200
GATGCCATACA	TCACAGGGCT	GTGCTTCATC	TCCATGGCCA	ATTACCTAGG	TTCTTTCTC	1260
AGCCCTCCAA	AAATTCACTG	GTCTGCCAGA	TCAAAACTCA	TIGAACCTTT	TTTTAACAAAG	1320
TTATTTCTTA	TGAGGGTCAT	GGATATCCCC	TATCTAAACT	TGGAAGGACG	AGACTTGCAG	1380
CCTAAACGTG	ATCATGTTCT	CCATCTGACA	TTCCCCAAAG	AATGGAAAAAC	CAGCGACCTT	1440
TACCAGCTTT	TCAGTGCCTT	TGGTAAACATT	CAGATATCCT	GGATTGATGA	CACATCACCA	1500
TTTGTTCCTC	TTAGCCAGCC	CGAGCAACTA	AAGATTGCTG	TCAATACCA	CAAATATGCA	1560
GAAAGCTATC	GGATCCAAAC	CTATGCTGAA	TATATGGGGA	GAACACAGGA	AGAGAAGCAG	1620
ATCAAAAGAA	AGTGGACTGA	AGATAGCTG	AAGGAGGCTG	ACACCAAAC	CTTAAACCCC	1680

CACTGGCATAC CCTACACCCT GCAGAAATCAC TATTACCGCA ACAATAAGTTT TACAGCTCCC	1740
ACGCACAGTAG GAAAGAGAAA TTTGAGTCCT AGTCRAGAGG AAGCTGGCTT GGAGGACCGA	1800
GTGTCAGGGG AGATTTCCGA CACTGAGCTT GAGCAGACCG ATTCTCTGTGC AGAGCCCCCTC	1860
TCAGAGGGAA CGAAAAAAGGC CAAGAAATTAA AAAAGAATGA AGAAGGAGCT TTCTCCAGCA	1920
CGAACGCATCT CGAACGAAACAG CCCTGCCACA CTCTTGAAAG TTCTGTGACAC ATGGTAACCA	1980
AGACCTGAGG CGACGAAACC GCTGGTGTG TCGCTGTGAC CAAGACCCCG CTGGCACATT	2040
TGGAAGCCGC ACTGTATTTA ACTTAATCAA ATGTCGTATG GGAGGGGTTG GAAACCAAGT	2100
TGTCTCTGG GGGGGAGAAA ACAGGTTTTA TTTTGTGGC TGTGGTTTT TCCCCCTTTT	2160
AATCTAATCG CCTGTGACA TTGACACTCA TCACCGTTGT AGGCTGTCT ATGATGTGTAC	2220
GTGCTTAACC AGTGAATTCC GTGTTGCTCT TGTGAGGCCCT TTCTGTCTAT GACCCAGTGT	2280
GCTTAAGAAC CTGGCTGATG CGGAGTGTG GCTGTGAAT CTGCAAAAAG AGCTGACATT	2340
CCAGCTGCTG TGATCATGAA TTGGGGCTG TACTGTCTG CCTGTGCATC TTCTCGCACT	2400
GAGATTTGA GGCAGTTGCA GCCCTCGGTT AGTCTCCAG TGAGAAAARTC GGTTGTGCCT	2460
CCCTGCTTCC CACCATAGCT GCCTGAAAC ATGACGCTCT CAAGCTGTCT TTCTCTTCAG	2520
GAAGATGTCC ACTCATGCC ACCCATGAGA GGGCTGCGG TATGCCCTGG CCTTTGGGCA	2580
TATTTATGTA GAGTTCTTCTT CTCTCTAACAG GTGACTTTCT CATGGGGGAT GTACGAGTAA	2640
AAAGGTTAAC TTCTGTTCTT ATGCGTGGCGG CTGCTGTTCAC TTCCAGAGT CTCTGTTCTGT	2700
TTGTTGGAT CGCGGTCTCG GGGTACCGCA GCGTGTGTGC CTACCGTGTCT GTGTGTGTGT	2760
GTGTGTGTGT GTGTGTGTGT GTGTGTGTGA AATCTGCAAT ATCTACAAACA TGTCCCAGCC	2820
CATTCTCCGT TGAACACAGAT CACAGCAACG ACAACGCTC ATGGCGCTGC TTGCTCCAC	2880
CCGCTTCAGA TAGATCATTG TTAGATATT CACATTITG TATGGTGGAA ATAAAAATGA	2940
AAAATGTATT TCCAAAAGAT GAAAATTAAA AACATTTCAGA TAGGAAAAAA AAAAAAAAAA	3000
AA	2

## (2) INFORMATION FOR SEQ ID NO: 13

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 12
  - (B) TYPE: Amino acid sequence
  - (C) STRANDEDNESS: Single
  - (D) TOPOLOGY: Linear
  
- (ii) MOLECULE TYPE: Peptide
  
- (v) FRAGMENT TYPE: Internal fragment
  
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13

ADFFAIGFSG IS

12

## (2) INFORMATION FOR SEQ ID NO: 14

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 15
  - (B) TYPE: Amino acid sequence
  - (C) STRANDEDNESS: Single
  - (D) TOPOLOGY: Linear
  
- (ii) MOLECULE TYPE: Peptide
  
- (v) FRAGMENT TYPE: Internal fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13

LVIGHNMLLV MHTVH

15

(2) INFORMATION FOR SEQ ID NO: 14

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16
- (B) TYPE: Amino acid sequence
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(v) FRAGMENT TYPE: Internal fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14

SEQLHEAGYA YITGLC

16

(2) INFORMATION FOR SEQ ID NO: 15

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12
- (B) TYPE: Amino acid sequence
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(v) FRAGMENT TYPE: Internal fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15

CDFVAIFFLG LD

12

(2) INFORMATION FOR SEQ ID NO: 16

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 15
  - (B) TYPE: Amino acid sequence
  - (C) STRANDEDNESS: Single
  - (D) TOPOLOGY: Linear
- (ii) MOLECULE TYPE: Peptide
- (v) FRAGMENT TYPE: Internal fragment
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16

LVVGHNSLLA MYMYH

15

## (2) INFORMATION FOR SEQ ID NO: 17

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 16
  - (B) TYPE: Amino acid sequence
  - (C) STRANDEDNESS: Single
  - (D) TOPOLOGY: Linear
- (ii) MOLECULE TYPE: Peptide
- (v) FRAGMENT TYPE: Internal fragment
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17

ENVYHNAGFS YVTGEV

16

## (2) INFORMATION FOR SEQ ID NO: 18

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 12
  - (B) TYPE: Amino acid sequence
  - (C) STRANDEDNESS: Single
  - (D) TOPOLOGY: Linear
- (ii) MOLECULE TYPE: Peptide

(v) FRAGMENT TYPE: Internal fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18

ADFVAILMTG VT

12

(2) INFORMATION FOR SEQ ID NO: 19

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15
- (B) TYPE: Amino acid sequence
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(v) FRAGMENT TYPE: Internal fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19

LIVGHNCFLI AHVYS

15

(2) INFORMATION FOR SEQ ID NO: 20

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16
- (B) TYPE: Amino acid sequence
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(ii) MOLECULE TYPE: Peptide

(v) FRAGMENT TYPE: Internal fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20

AGGKHEAGYA FMTGCI

16

## (2) INFORMATION FOR SEQ ID NO: 21

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 12
  - (B) TYPE: Amino acid sequence
  - (C) STRANDEDNESS: Single
  - (D) TOPOLOGY: Linear
- (ii) MOLECULE TYPE: Peptide
- (v) FRAGMENT TYPE: Internal fragment
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20

GTLVIAIAFVS LQ

12

## (2) INFORMATION FOR SEQ ID NO: 22

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 15
  - (B) TYPE: Amino acid sequence
  - (C) STRANDEDNESS: Single
  - (D) TOPOLOGY: Linear
- (ii) MOLECULE TYPE: Peptide
- (v) FRAGMENT TYPE: Internal fragment
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22

VFVGHGLNNF KHINI

15

## (2) INFORMATION FOR SEQ ID NO: 23

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 16
  - (B) TYPE: Amino acid sequence
  - (C) STRANDEDNESS: Single
  - (D) TOPOLOGY: Linear

- (ii) MOLECULE TYPE: Peptide
- (v) FRAGMENT TYPE: Internal fragment
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23

QEGNHDSIEA HTALIL

16

## (2) INFORMATION FOR SEQ ID NO: 24

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 12
  - (B) TYPE: Amino acid sequence
  - (C) STRANDEDNESS: Single
  - (D) TOPOLOGY: Linear
- (ii) MOLECULE TYPE: Peptide
- (v) FRAGMENT TYPE: Internal fragment
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24

FPAIALTFVR TR

12

## (2) INFORMATION FOR SEQ ID NO: 25

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 15
  - (B) TYPE: Amino acid sequence
  - (C) STRANDEDNESS: Single
  - (D) TOPOLOGY: Linear
- (ii) MOLECULE TYPE: Peptide
- (v) FRAGMENT TYPE: Internal fragment
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25

TKFLHAGSEL EVFLN

15

## (2) INFORMATION FOR SEQ ID NO: 26

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 15
  - (B) TYPE: Amino acid sequence
  - (C) STRANDEDNESS: Single
  - (D) TOPOLOGY: Linear
- (ii) MOLECULE TYPE: Peptide
- (v) FRAGMENT TYPE: Internal fragment
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26

ERQEWAAAVW YLLPI

15

## (2) INFORMATION FOR SEQ ID NO: 27

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 12
  - (B) TYPE: Amino acid sequence
  - (C) STRANDEDNESS: Single
  - (D) TOPOLOGY: Linear
- (ii) MOLECULE TYPE: Peptide
- (v) FRAGMENT TYPE: Internal fragment
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27

APVFAFTTDS LD

12

## (2) INFORMATION FOR SEQ ID NO: 28

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 15
  - (B) TYPE: Amino acid sequence

05624032 20080606

- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

- (ii) MOLECULE TYPE: Peptide
- (v) FRAGMENT TYPE: Internal fragment
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28

LKVGQNLKYR GILAN

15

## (2) INFORMATION FOR SEQ ID NO: 29

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 15
  - (B) TYPE: Amino acid sequence
  - (C) STRANDEDNESS: Single
  - (D) TOPOLOGY: Linear
- (ii) MOLECULE TYPE: Peptide
- (v) FRAGMENT TYPE: Internal fragment
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29

EEAGRAAEAD VTLQL

15